



Catalase-coupled gold nanoparticles: Comparison between the carbodiimide and biotin–streptavidin methods

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ABSTRACT

The use of proteins for therapeutic applications requires the protein to maintain sufficient activity for the period of in vivo treatment. Many proteins exhibit a short half-life in vivo and, thus, require delivery systems for them to be applied as therapeutics. The relative biocompatibility and the ability to form functionalized bioconjugates via simple chemistry make gold nanoparticles excellent candidates as protein delivery systems. Herein, two protocols for coupling proteins to gold nanoparticles have been compared. In the first, strong biomolecular binding between biotin and streptavidin was used to couple catalase to the surface of gold nanoparticles. In the second protocol the formation of an amide bond between carboxylic acid-coated gold nanoparticles and free surface amines of catalase using carbodiimide chemistry was performed. The stability and kinetics of the different steps involved in these protocols were studied using UV–visible spectroscopy, dynamic light scattering, and transmission electron microscopy. The addition of mercapto-undecanoic acid in conjugation with (*N*-(6-(biotinamido)hexyl)-3'-(2'-pyridyldithio)-propionamide increased the stability of biotinylated gold nanoparticles. Although the carbodiimide chemistry-based bioconjugation approach exhibited a decrease in catalase activity, the carbodiimide chemistry-based bioconjugation approach resulted in more active catalase per gold nanoparticle compared with that of mercapto-undecanoic acid-stabilized biotinylated gold nanoparticles. Both coupling protocols resulted in gold nanoparticles loaded with active catalase. Thus, these gold nanoparticle systems and coupling protocols represent promising methods for the application of gold nanoparticles for protein delivery.

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1. Introduction

Carrier systems of nanoscale dimensions, such as liposomes, polymeric particles, and microemulsion droplets, are being widely studied for the delivery of various biomolecules [1,2]. In the case of proteins/enzymes an important requirement for their immobilization to various carrier systems is that the protein should remain in its active form and be able to carry out the necessary functions efficiently. Although physical adsorption of proteins via hydrophobic and electrostatic interactions is experimentally simple, the loss of protein/enzyme from the carrier once in contact with the in vivo environment makes chemically mediated immobilization via covalent bonding attractive to increase the half-life of proteins for in vivo therapeutic applications. Additionally, irreversible covalent binding generally leads to high levels of surface coverage. The easy synthesis of inorganic nanoparticles, their high surface to volume ratio, and the ability to control their size prove vital in immobilizing proteins over their surfaces for therapeutic applications [3]. Added advantages of gold nanoparticles (GNPs) include biocom-

patibility, relative non-toxicity, and the ability to form functionalized bioconjugates via simple chemistry [4]. Some of the in vivo therapeutic applications of functionalized GNPs include tumor necrosis factor delivery [5], treatment of colon carcinomas with Paclitaxel-grafted gold colloids [6], cellular drug delivery [7–9], gene therapy [10–14], thermoablative therapy [15,16], and drug release [17–19]. With the advent of such successful applications, GNPs could also prove to be suitable carriers for therapeutic protein delivery.

GNPs serve as excellent candidates for protein bioconjugation, because they readily react with the amino and cysteine thiol groups of proteins. Unlike polymeric systems, which prove effective in reducing the proteolytic degradation and deactivation of the therapeutic reagent [2,20–22], proteins conjugated to GNPs are directly exposed to their environment and potential proteolysis. Although this exposure is a potential drawback, it overcomes substrate diffusion/accessibility limitations of encapsulated systems. One potential method to overcome proteolysis is to use polymeric (e.g. poly(ethylene glycol) (PEG)-based) brushes in conjunction with proteins, which can minimize the access of proteolytic enzymes to the surface of particles. Further, the inherent properties of their metal core make GNPs an effective drug carrier platform for combinatorial

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diagnosis through heavy metal imaging, along with protein therapy [23]. Also, issues of rapid *in vivo* therapeutic clearance, poor inherent localization at targeted sites, and the simultaneous protection of untargeted sites from undesired reactions can be overcome through the functionalization of GNPs with polymeric brushes, such as PEG [24], and biomolecular targeting agents [3,25]. Several proteins, such as insulin, pepsin, glucose oxidase, horseradish peroxidase, xanthine oxidase, fungal protease, etc., have been directly conjugated to GNPs [2,26–30]. In most cases this strategy alters the conformational structure and active center of the protein, similarly to physical adsorption, thereby causing a reduction in activity [31]. Herein we report and compare two simple procedures for coupling a model enzyme catalase to GNPs. Catalase catalyzes the conversion of hydrogen peroxide, a harmful oxidizing agent, to water and molecular oxygen. Its antioxidant properties have been studied extensively for potential effectiveness in antioxidant therapy. However, catalase undergoes rapid elimination from the bloodstream and demonstrates poor intracellular delivery [32]. Fig. 1 shows a schematic representation of the two approaches utilized in this study for the coupling of catalase to GNPs. The first involves the biotinylation of both the GNP and catalase and then coupling them together using a streptavidin cross-linker. The second method uses carbodiimide chemistry to form amide bonds between carboxylic acid-coated GNPs and amino groups of catalase. The first method takes advantage of the high affinity of biotin for streptavidin to form a biotin–streptavidin complex, which is among the strongest interactions in biology. The advantage of using carbodiimide chemistry is that it employs mild reaction conditions, endowing considerable versatility of bioconjugation for a wide variety of protein molecules. The common feature of both procedures is exploitation of the attachment of thiol molecules to the surface of GNPs via thiol–gold chemistry. Characterization of the coupling kinetics, stability of the modified particle, and activity of the catalase bound to GNPs were done using UV–visible spectroscopy, dynamic light scattering, and transmission electron microscopy (TEM). With these simple methods of bioconjugation protein-coupled GNPs could be useful for the delivery of proteins in biomedical applications.

2. Experimental section

2.1. Synthesis of biotinylated and carboxylic coated GNPs

Monodispersed GNPs were prepared using the Turkevich reduction of gold salts [33]. Briefly, a 1 mM aqueous solution of

chloroauric acid (HAuCl_4) was boiled under stirring. To this solution was added 3 mM trisodium citrate in water to reduce HAuCl_4 , producing GNPs of sub-nanometer size. Tween 20 at 38 mg ml^{-1} was added to a 1 mg ml^{-1} final concentration to stabilize the particles for further functionalization. To incorporate biotin into the GNP a 4 mM stock solution of (*N*-(6-(biotinamido)hexyl)-3'-(2'-pyridylidithio)-propionamide (biotin-HPDP) (Soltech Ventures) in dimethyl sulfoxide (DMSO) was added to the Tween stabilized GNPs so that the final concentration of biotin-HPDP was 0.5 mM and the mixture incubated at room temperature for 4 h. The biotinylated GNPs were then dialyzed using 12 kDa molecular weight cut-off tubing against water to remove excess unreacted biotin-HPDP and stored for further use.

For the carbodiimide chemistry coupling method carboxylic groups were introduced on the surface of GNPs by adding a 10 mM stock solution of mercapto-undecanoic acid (MUDA) in ethanol (Asemlon) to Tween stabilized GNPs to a final concentration of 0.5 mM, followed by stirring the solution for 12 h at room temperature. The biotinylated GNPs and MUDA-coated GNPs were dialyzed against water to remove free MUDA. Additionally, GNPs containing varying concentrations of surface biotin were synthesized using MUDA as the stabilizing reagent. For this the final concentration of MUDA was fixed at 0.5 mM, while biotin-HPDP was added at 50 μM for 1:10 biotin:MUDA GNPs and at 25 μM for 1:20 GNPs. Both biotin-HPDP and MUDA were added to the stabilized GNPs at the same time, and the reaction was allowed to proceed for approximately 12 h.

2.2. Biotinylation of catalase

The biotinylation of catalase was carried out using succinimide chemistry. Briefly, biotin-*N*-hydroxysuccinimide (BNHS) (Vector Laboratories) at 25 mg ml^{-1} in DMSO was added to a 2 mg ml^{-1} catalase⁻¹ (Calbiochem) solution in 100 mM HEPES buffer, pH 8.0, to a final concentration of BNHS of 10 wt.% of the enzyme to be biotinylated. The mixture was stirred occasionally for 3 h, after which 10 μl of 16.4 M ethanolamine was added to stop the reaction by reacting with any free biotin-NHS. The biotinylated catalase was dialyzed against 1 l of 100 mM HEPES buffer, pH 8.0, with three buffer changes.

2.3. Coupling of catalase to the GNP surface via biotin–streptavidin binding

Purified biotinylated GNPs were dialyzed against HEPES prior to catalase coupling. Biotinylated GNPs in HEPES were treated with a

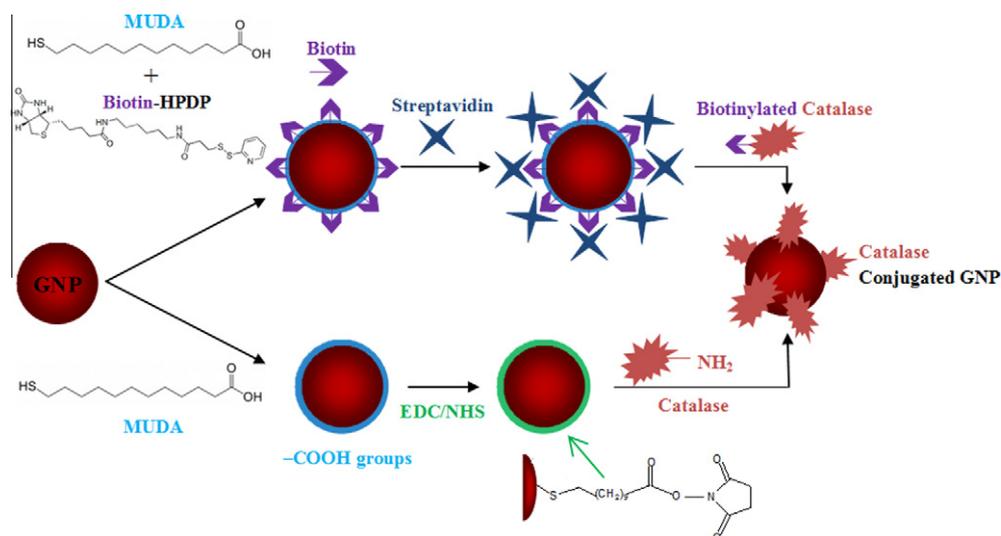


Fig. 1. Schematic representation of the two approaches involved in the coupling of catalase to gold nanoparticles.

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